DISTRIBUTION AND ABUNDANCE OF BENTHIC FORAMINIFERA ALONG THE INTERTIDAL AREA OF PENANG ISLAND

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by

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ºC	Degree Celsius
ppt	Part per thousand
µm	Micrometer
mm	Millimeter
cm	Centimeter
m	Meter
m^2	Meter square
cm^3	Centimeter cubic
ml	Milliliter
mg/L	Milligram per liter
%	Percent

TABURAN DAN KELIMPAHAN FORAMINIFERA BENTIK SEPANJANG KAWASAN PASANG SURUT DI PULAU PINANG

ABSTRAK

Taburan dan kelimpahan foraminifera bentik di kawasan intertidal Pulau Pinang telah dikaji. Lima tapak kajian telah dipilih iaitu Batu Feringghi, Jelutong, Pulau Betong, Teluk Aling dan Teluk Kumbar. Persampelan foraminifera telah dijalankan dari tiga zon pasang surut rendah, tengah dan atas. Sejumlah 375 sampel sedimen dikumpulkan dua bulan sekali antara Mei 2015 dan Januari 2016. Parameter persekitaran (pH, kemasinan dan suhu) diukur secara *in-situ*. Jirim organik dianalisis berdasarkan kehilangan kaedah pembakaran (LOI) dan saiz partikel dilakukan berdasar dari kaedah Bale dan Kenny (2005). Sebanyak 9 genera foraminifera telah dikenalpasti iaitu *Ammonia, Elphidium, Quinqueloculina, Pseudorotalia, Bolivina, Nonionellina*, *Haynesina* dan *Spiroloculina.* Secara umumnya, taksa toleransi-tekanan, *Ammonia* (64.11%) dan *Elphidium* (14.23%) menguasai himpunan di kesemua tapak kajian. Indeks kepelbagaian menunjukkan bahawa kawasan pasang-surut Jelutong mempunyai himpunan yang pelbagai (H '= 1.61; D = 0.77) dan Teluk Kumbar menunjukkan kepelbagaian terendah (H' = 0.43; D = 0.33). Analisis kluster mengklasifikasikan tapak kajian kepada dua kumpulan, masing-masing dipengaruhi oleh keadaan persekitaran yang berbeza. Kumpulan A (Batu Feringghi, Teluk Aling dan Teluk Kumbar) dicirikan oleh pantai berpasir (saiz partikel = 1 mm ~ 250 μm) dengan pH (7.72), kemasinan (34.00 ppt) dan suhu (33.5^{0} C) (ANOVA, P <0.05) yang tinggi. Kumpulan ini mempunyai kelimpahan foraminifera yang lebih tinggi tetapi rendah dalam kepelbagaian. Sementara itu, kumpulan B (Jelutong dan Pulau Betong) terdiri daripada tapak kajian yang tidak berpasir dan menunjukkan kandungan jirim organik yang tinggi (3.04%; ANOVA, $p < 0.05$). Kumpulan ini dicirikan dengan min kelimpahan yang rendah tetapi tinggi dalam kepelbagaian. Daripada analisis korelasi Pearson,menunjukkan tujuh genera iaitu *Bolivina*, *Pseudorotalia, Nonionellina, Elphidium, Quinqueloculina, Haynesina* dan *Spiroloculina* berkolerasi positif dengan pasir kasar (1 mm), pasir sederhana (630 μm), pasir campuran (425 μm) dan pasir halus (250 μm). Hanya pasir halus (250 μm) yang menunjukkan korelasi dengan semua genus foraminifera. Indeks FORAM (FI = 1.0 ~ 2.30) dan indeks *Ammonia-Elphidium* (AEI = 61 ~ 89) diperolehi dalam kajian ini mencadangkan bahawa semua tapak kajian yang dipilih dianggap sebagai berada di bawah tekanan dan terganggu (kecuali Teluk Aling). Kajian juga menyimpulkan bahawa foraminifera boleh digunakan sebagai penunjuk bio mesra ekonomi untuk pemantauan keadaan pesisir pantai di Malaysia.

DISTRIBUTION AND ABUNDANCE OF BENTHIC FORAMINIFERA ALONG THE INTERTIDAL AREAS IN PENANG ISLAND

ABSTRACT

The distribution and abundance of benthic foraminifera were studied in the intertidal areas of Penang Island. Five sites were selected namely Batu Feringghi, Jelutong, Pulau Betong, Teluk Aling and Teluk Kumbar. The samples of foraminifera were collected from three different intertidal zones i.e. low, middle and upper zones. A total of 375 sediment samples were collected bimonthly between May 2015 and January 2016. Environmental parameters (i.e. soil pH, salinity and temperature) were measured *in-situ*. Organic matter content was analyzed based on loss of ignition method (LOI) and particles size was determined based on Bale and Kenny (2005) method. A total of 9 genera of foraminifera were identified which include *Ammonia, Elphidium, Quinqueloculina, Pseudorotalia, Bolivina, Nonionellina, Haynesina* and *Spiroloculina*. Generally, the stress-tolerant taxa, *Ammonia* (64.11 %) and *Elphidium* (14.23%) dominated the assemblage at all sampling sites. Diversity indices showed that the intertidal area of Jelutong had the most diverse assemblage (H' =1.61; D = 0.77) and Teluk Kumbar (H'= 0.43; D = 0.33) had the lowest foraminiferal assemblages. The cluster analysis classified the sites into two groups, of which each group was influenced by different environmental conditions. Group A (Batu Feringghi, Teluk Aling and Teluk Kumbar) was characterized as sandy (grained-size = 1 mm ~ 250 µm) with high pH (7.72), salinity (34.00 ppt) and temperature 33.5^{0} C) (ANOVA, $p<0.05$). This group had a higher foraminiferal abundance but relatively low in diversity. Group B (Jelutong and Pulau Betong) consisted of non-sandy beach sampling sites and showed significantly high organic matter content (3.04%, ANOVA, $p < 0.05$). This group was characterized by low mean abundance but high in diversity. Pearson's correlation analysis showed that seven genera i.e. *Bolivina, Pseudorotalia, Nonionellina, Elphidium, Quinqueloculina, Haynesina* and *Spiroloculina* were positively correlated with coarse sand (1 mm), medium sand (630 µm), mixture sand (425 µm) and fine sand (250 µm). Only fine sand (250 µm) showed both positive and negative correlations with all the genus of foraminifera. The values of FORAM index (FI = 1.0 ~ 2.30) and *Ammonia-Elphidium* index (AEI = 61 ~ 89) obtained in this study suggested that all selected sites were regarded as under the stressed condition and disturbed. The study also concluded that foraminifera can be used as economically-friendly bio-indicators for monitoring coastal areas in Malaysia.

CHAPTER 1 - INTRODUCTION

Foraminifera are unicellular organisms, possessing cytoplasm either with one or more nuclei covered by shells known as tests (Murray, 2006). They are believed to have been existed since the Cambrian era and in the present day, these ameboid-like organisms are found living in the ocean. Numerous types of tests exist, and are made up of calcareous, porcelains, hyaline and agglutinated. In general, these tests determine their classes and species, as well as illustrate the habitat and survival level of the foraminifera (Scott et al., 2001). Several morphological features such as chamber arrangement, test structure and aperture have been used for identification and classification.

At present, more than 40,000 species of foraminifera have been recorded. Benthic foraminifera reside in most of the marine environment from the deepest part of the ocean up to the surface of the marine waters (Camacho et al, 2015). Planktonic foraminifera reside in the water column with a higher rate of movement. They are sensitive to slight changes of environmental parameters such as salinity, pH and temperature. They are also highly-responded to both anthropogenic and natural changes, for instance, a stressful environment will result in lower variability within populations (Takata et al., 2012).

Due to their immediate response to environmental changes, several authors have suggested foraminifera as environmental indicators using two main indices namely, the *Ammonia-Elphidium* index (AEI) (Sen Gupta et al., 1996) and FORAM index (Hallock et al., 2003). The *Ammonia-Elphidium* index has been used to measure the hypoxia level in the sediments. Meanwhile, the FORAM index is used to measure the quality of the water and sediments in the marine environment.

Both natural and anthropogenic stressors such as temperature and siltation are among factors causing the changes to the intertidal zones. Degradation of the coastal zones and intertidal zones; and the aquatic ecosystem itself are caused by the accumulation of the pollutant. The increased levels of the pollutants over the year degrade the habitats for certain benthic faunas, particularly those with high intolerance to the pollution.

In Malaysia, studies on foraminifera in Malaysia was pioneered by Mohamed et al. (2005) who focused on the distribution and assemblages of the agglutinate foraminifera in the estuary Sedili River situated in the southern part of Malaysia. Several other studies were done by Hawkes et al. (2007) and Husain et al. (2007) on the distribution of foraminifera in the mangrove and estuarine environment. Meanwhile, Minhat et al. (2013) and Khairun et al. (2014) observed the distribution of foraminifera in coastal waters of Penang Island. In 2014, the potential use of foraminifera as bio-indicators to monitor the ecosystem health has been initiated by Minhat et al. (2014).

Penang Island is located in the north-western of Peninsular Malaysia, comprising both mainland and island parts. Penang Island is currently experiencing urbanization, migration and increasing population rate, and this phenomenon has resulted in ecological pressure (Masron et al., 2012). Based on the recent census, Penang is among the state that has high population density in Malaysia with approximately 130.77 people/km^2 (Department of Statistic Malaysia). With an area of 293km^2, Penang Island has extremely limited land areas compared to the rapidly growing population. Due to the population pressure, many coastal land reclamation projects have been carried out to meet the demand. Therefore, it is crucial to identify the sediment quality of the marine environment around Penang Island. The use of benthic organisms as bio-indicators is one of the alternatives to monitor the status of the marine ecosystem.

In this study, benthic foraminifera were used as bio-indicators to monitor the health of the Penang Island ecosystem due to their profound ability to assess the sediment quality using indexes (i.e. *Ammonia-Elphidium* index and Foraminifera index). The distribution of foraminifera in the north, south, east and west sides of the Penang Island was investigated. The physical parameters and assemblages of foraminifera were used to determine the ecosystem health of the marine environment of the island.

Therefore, the aims of the study were:

1. To study the composition and distribution of foraminifera in the intertidal areas of Penang Island
2. To determine the correlation between environmental parameters and distribution of foraminifera
3. To determine the reliability of AEI and FORAM indices to measure the degree of stress in the intertidal areas

CHAPTER 2 - LITERATURE REVIEW

2.1 Foraminifera: An Overview

The benthic foraminifera are considered as the most outstanding protozoa that live within the marine sediments (Langezaal et al, 2003). They are ubiquitous and are regularly found in the marine environment. Foraminifera have existed since in the era of Cambrian and continuously evolve during Phanerozoic until today (Wray et al., 1995).

Foraminifera are classified as protozoans as they are single-celled, comprising of cytoplasm with one or more nuclei (Murray, 2006). Hesemann (2009) stated that foraminifera are protozoa with an enclosed amoeboid structure such as nucleus, vacuoles and cytoplasm. The cytoplasm is protected by their external shell known as a test that builds up from the chambers of the organism (Ayadi et al., 2015).

2.2 Classification of foraminifera

Loeblich and Tappan (1978; 1992) classified foraminifera based on the complexity of their tests and life cycle. The test of foraminifera is used to primarily classify them, mainly based on its characteristics, chamber arrangement and the type of aperture (Debenay, 2012). Three different types of tests have been recognized thus far i.e. organic matter, agglutinated and a made up of calcium carbonate secretion (Murray, 2006). Agglutinated tests are made up of surrounding sediments or organic particles such as quartz, silt and many other minerals in the sediments. Meanwhile, calcium carbonate is the main component of the calcareous shell.

2.3 Morphological characteristic of foraminifera

The most significant characteristic of foraminifera is the presence of the test that protects the nucleus from predators, provides shelter from threatening external factors, favors in reproduction process and regulates the organism’s buoyancy (Murray, 2006).

Most foraminiferal species are built with multilocular chamber arrangement known as multilocular (Debenay, 2012). The test shapes of the multi arranged chamber are varied such as serial, spiral or milioline arranged chambers (Hesemann, 2009) (Figure 2.1). Only a few are built with a single chamber known as unilocular. The single-chambered test is the simplest chamber that comes in shapes of tubular; radial branched, globose, irregular or flask-shaped.

The aperture is the first opening on the foraminiferal cavity towards the surrounding environment (Hottinger, 2006). It is a small opening where the final chamber is located and communicates with the outer environment (Debenay, 2012). Kaminiski et al. (2011) classified the arrangement of the chamber into uniserial, biserial and multiserial (Figure 2.2)

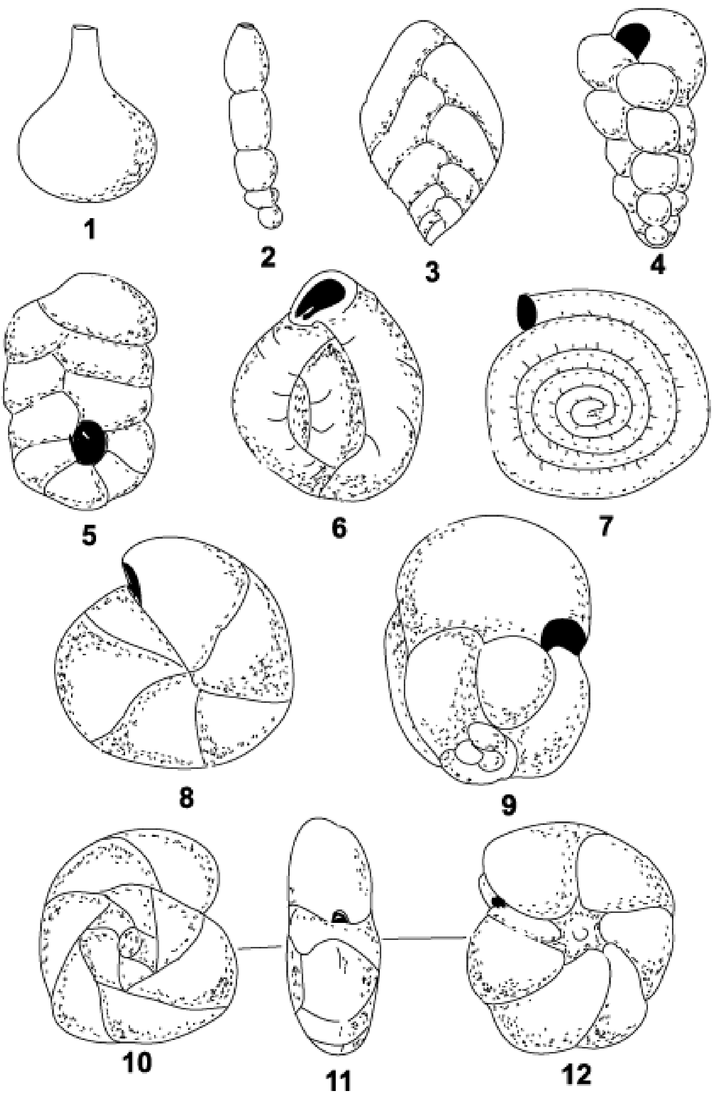

Figure 2. 1 shows the arrangement of chamber in foraminifera (1- single chamber, 2- uniserial, 3- biserial, 4-triserial, 5- planispiral to biserial, 6- millioline, 7- planispiral evolute, 8- planispiral involute, 9- streptospiral, 10-11-12, trochospiral (10- dorsal view, 11-edge view, 12- ventral view) Redrawn from Leoblich and Tappan (1964)

Source: http://www.ucl.ac.uk/GeolSci/micropal/foram.html

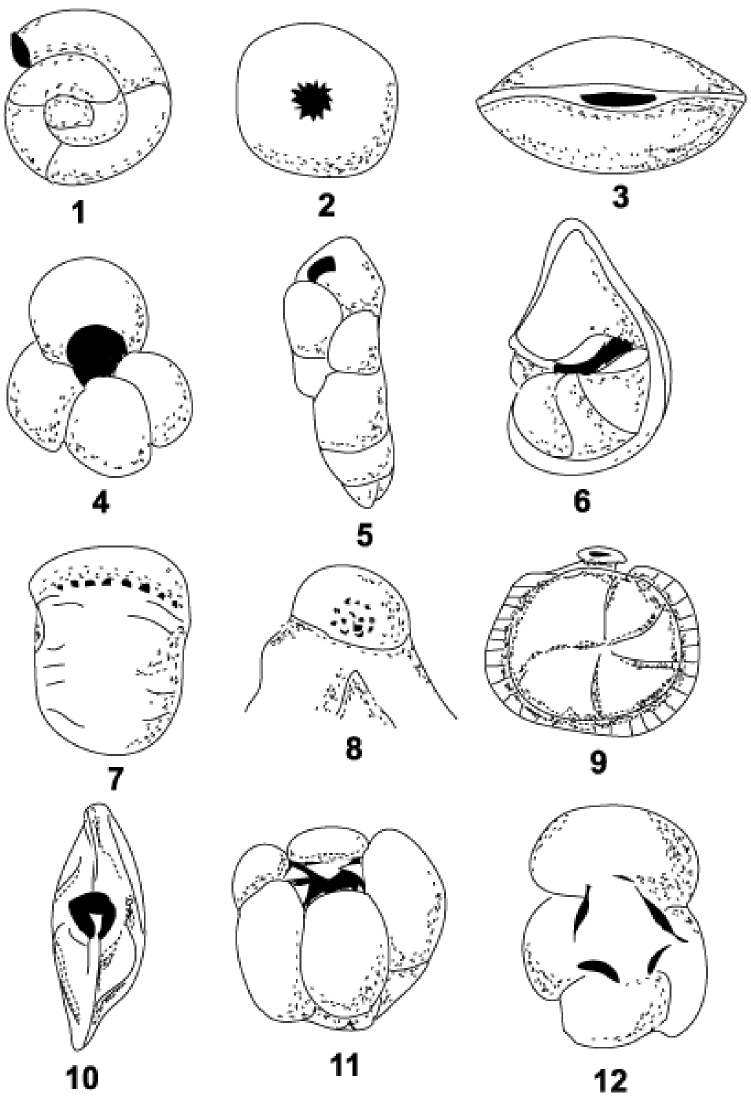

Figure 2. 2 type of aperture possessed by foraminifera (1- open end of tube, 2- terminal radiate, 3- terminal slit, 4- umbilical, 5-loop shaped, 6- interiomarginal, 7- interiomarginal multiple, 8- areal, 9- with phialine lip, 10- with bifid tooth, 11- with umbilical teeth, 12- with umbilical bulla) Redrawn from Loeblich and Tappan (1964)

Source: http://www.ucl.ac.uk/GeolSci/micropal/foram.html

2.4 Reproduction and life cycle of foraminifera

In general, reproduction of foraminifera differs according to species. They have a complex life cycle that comes with alternation of generations; between haploid and diploid generations (Debenay, 2012). Foraminifera undergo two different types of reproduction i.e. sexually and asexually (Murray, 2006). In asexual reproduction, they become dimorphic and outgrow a large proloculus known as megaspheric. While in the sexual reproduction, the resulting offspring are two small proloculus in a microspheric form. Instead of the dimorphic life cycle, foraminifera undergo trimorphic life cycle (Dettmering et al. 1998) (Figure 2.3)

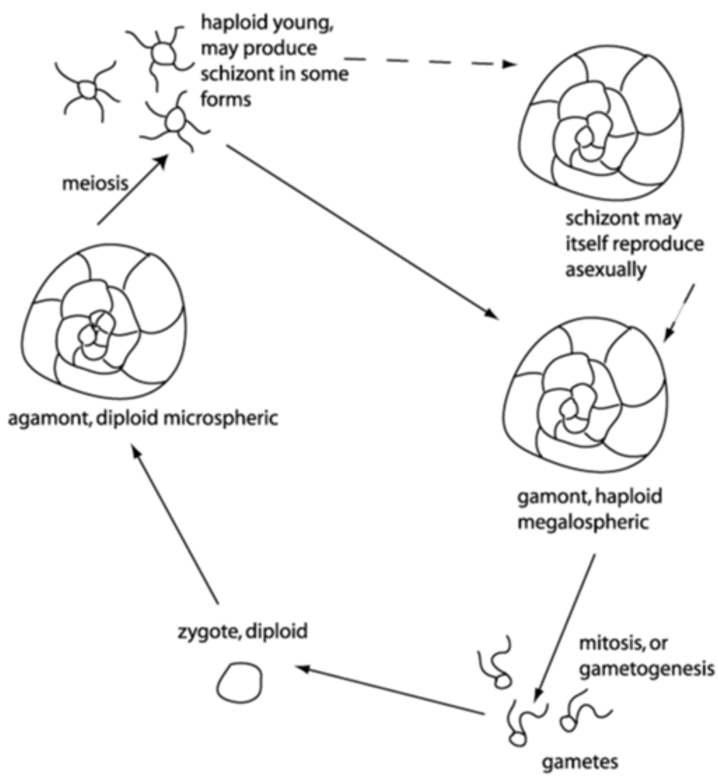

Figure 2. 3 life cycle and alteration of life between a haploid megalospheric form and a diploid microspheric form

Source: Source: http://www.ucl.ac.uk/GeolSci/micropal/foram.html

2.5 Ecology of Foraminifera

Apart of the marine environment, some foraminifera species are well-adapted to freshwater ecosystems (Holzmann & Pawlowski, 2002), estuary (Alve, 2003), wetlands and mangroves (Satyanarayana et al., 2014; Debenay et al., 2002).

The vertical distribution of benthic foraminifera is abundant on the surface of the sediment, particularly from 0 to 1 cm depth. However, the distribution decrease as they go lower below the ground, approximately at 20 cm depth (Dey et al., 2012).

2.5.1 Foraminifera in the intertidal areas

Albani et al. (1984) noted that the distribution of foraminifera is related to the vegetation of the environment. Many studies of foraminifera in the intertidal environment have been conducted to consider the potential use of intertidal foraminifera as an indicator for sea level changes (Kemp et al., 2013). For example, Horton (2006) examined a pattern of foraminiferal distribution in the British Isle concerning the sea-level reconstruction.

Intertidal foraminifera were also studied for paleoclimate; of which a study of the past environmental conditions i.e. temperature and future climate prediction (Scott et al., 2001). The study of benthic foraminifera in a shelf of Canadian Atlantic has illustrated the climate change where the bottom waters were warmer and more saline during the late Pleistocene-Holocene era (Scott et al., 1984).

Studies of intertidal benthic foraminifera are commonly associated with their vertical living distribution (Horton, 2005; Berkeley et al., 2007; Kemp et al., 2013), of which their distribution is generally associated with the tide level (Horton and Edwards, 2005). The ecology of foraminifera in the intertidal sediments and a taphonomic process (fossilization) was previously studied by Berkeley et al. (2007).

Based on the study, the taphonomic process was based on individual depth. They suggested that sediments at the upper layer alone were insufficient to represent the foraminiferal species input.

A large body of work has been conducted to study the sediments by using foraminifera after the incidence of the Indian Ocean Tsunami in December 2004. Mamo et al., (2009), for instance, studied the foraminiferal assemblages with respect to the pre- and post-tsunami incidents based on the post-tsunami sediments. Adding to this, a study of foraminifera has also been conducted along the Malaysia-Thailand Peninsular covering three sites in Thailand (Khuk Khak Beach, Tonsai village, Phi Phi Don and Somewhere Else Resort) and two sites in Malaysia (Kuala Triang, Langkawi and Sungai Burong, Penang). The study managed to differentiate the assemblages of foraminifera between pre- and post-tsunami incidents based on the content, species diversity and the size of the tests (Hawkes et al., 2007).

2.6 Zonation of intertidal areas

The intertidal zone or the littoral zone consists of three distinctive zones namely sub-littoral (lower zone), mid-littoral (middle zone) and supra-littoral (upper zone). The distance between each zone is varied depending on the length of the beach (Karleskint et al., 2010).

An intertidal area is an area where the seawater meets the land. It is divided into high tide, middle and low tide zones. In general, organisms that inhabit in this area possess particular characteristics to withstand and adapt to the harsh condition of the environment.

The zonation of the intertidal area can be distinguished by inundation frequency. For instance, the high tide zone is the region that is constantly submerged by water during high tide and is frequently exposed to the air compared to water. Meanwhile, the middle zone is the region that is submerged by water and exposed to the air at an equal period. The low tide zone, on the other hand, is the region where it is submerged by seawater at all times and is only exposed to the air during extreme low tides i.e. mean lower low water.

The zonation is typically defined and classified based on inhabiting flora. For example, Kemp et al. (2013) characterized the zonation of the salt marsh in the intertidal area of New Jersey, USA based on the different species of cord grass that inhabited each zone at the area.

2.7 Foraminifera as bio-indicators

Numerous presence of foraminifera in the sediments and their widespread distribution could reflect the environmental quality (Aloulu et al., 2011) and can be used as bio-indicators for water circulation and sediment transport (Debenay, 2012).

In addition, the sampling of foraminifera is cost-effective and environmentally safe (Capotondi et al., 2014). Foraminifera have been used to assess the environmental health in shrimp farms (Debenay et al., 2009) and have been widely used as tools to monitor ecological and environmental changes and bio-indicators for marine environments (Al- Enezi and Frontalini, 2015). Foraminifera are selected as proxies because of their widespread distribution in the marine ecosystem (Culver et al., 2012). Foraminifera can also be used as palaeoenvironmental indicators following a study made by Mamo et al. (2009) on post-tsunami sediments in the Philippines.

Hallock et al. (2003) have proposed the Foraminifera in Reef Assessment and Monitoring (FORAM) Index as an indication whether the conditions of the benthic environments favor calcifying and algal-symbiotic organisms (Koukoisioura et al., 2011). Two opportunistic species i.e. *Ammonia* and *Elphidium* have been used to determine *Ammonia-Elphidium* Index (AEI). This index is used to measure the oxygen content in the sediments, of which the presence of stress-tolerant taxa i.e. *Ammonia* reflects the hypoxia condition in the sediments (Sen Gupta and Platon, 2005).

2.8 Past studies on Foraminifera in Asia

Studies on foraminifera in Asia were previously initiated by Khare (1927) in Indian waters which focused on their taxonomy and ecology. Two decades later, Jian et al. (1999) pioneered a study in the South China Sea including Southeast Asia such as Indonesia and Thailand. In 1998, a study of deep sea foraminifera was conducted in Mount Pinatubo, the Philippines following the massive volcanic eruption in June 1991 (Hess et al., 2001).

In Malaysia, studies on foraminifera are largely on the distribution rather than their role as bio-indicators. Culver et al. (2012), for example, focused on the distribution of foraminifera in estuary and lagoon in Setiu, Terengganu as well as the impact of aquaculture sectors on foraminifera and the sediments. Suriadi (2011) conducted a study of foraminiferal assemblages in the mangrove sediments of Kemaman and Tumpat, focusing on the relationship between environmental parameters and the distribution of foraminifera.

In the northern part of Malaysia, Wan Saelan (2011) conducted a study on foraminiferal assemblages in mangroves area of Larut Matang, Perak. Three years later, Satyanarayana et al. (2014) conducted a study at mangrove areas of Kapar and Matang, Perak and found that the areas were highly dominated by calcareous and agglutinated species. In Penang, studies on the distribution of benthic foraminifera along the coastal water of Penang Island were done by Minhat et al. (2013) and Khairun et al. (2014) where 14 genera of benthic foraminifera were recovered in their studies.

CHAPTER 3 - MATERIALS AND METHOD

3.1 Sampling Sites

Sampling was conducted bimonthly from March 2015 to January 2016 at five selected sites i.e. Pulau Betong, Teluk Kumbar, Batu Feringghi and Jelutong and Teluk Aling. (Figure 3.1). These sampling site were selected based on the degree of human activities and representative of west, east, north and south side of Penang island. The location of each sampling site was recorded by using the Global Positioning System (GPS) (Garmin GPS map 60CSx). The coordinates of each site are listed in Table 3.1.

Table 3. 1 The GPS coordinates for all 5 intertidal sampling sites.

Sampling site	**Coordinates**
Teluk Aling	N 05°28.078' E 100° 12.009´
Teluk Kumbar	N 05°16.911' E 100° 12.721’
Batu Feringghi	N 05°28.456' E 100° 14.811’
Pulau Betong	N 05°18.537' E 100° 11.619’
Jelutong	N 05°22.882' E 100° 19.090’

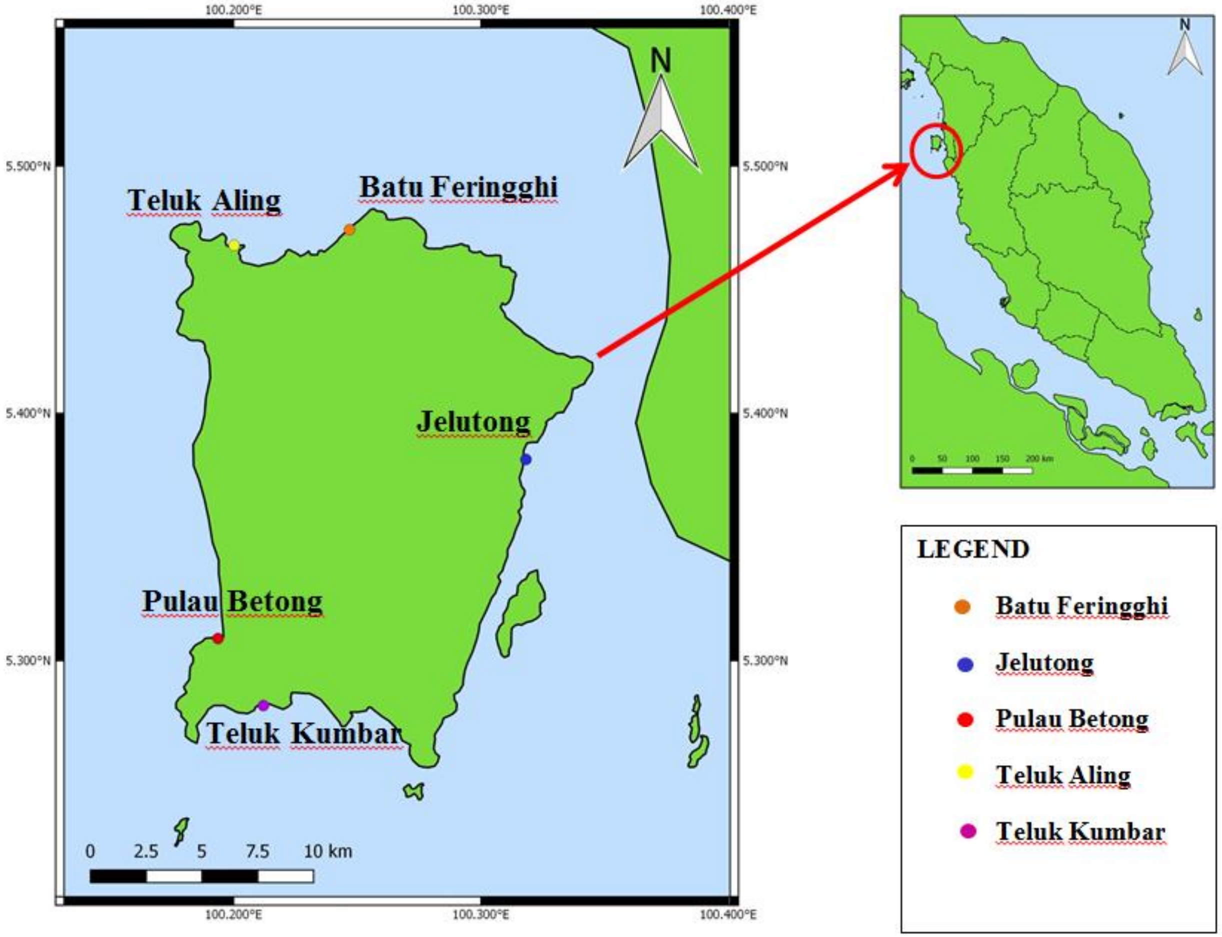

Figure 3. 1 Maps showing the five sampling sites

3.1.1 Pulau Betong

Pulau Betong is a fishing village located on the southwest district of Penang Island. The intertidal area has an extensive mudflat and is exposed to the sun especially during low spring tide. It is surrounded by a mangrove forest and a river known as Pulau Betong River that flows along the intertidal area. The beach gradient was 49º.

Plate 3. 1 The intertidal area of Pulau Betong

3.1.2 Teluk Kumbar

Plate 3.2 illustrates the sampling site of Teluk Kumbar. Teluk Kumbar is situated in the south area of the Penang Island and its sandy beaches are popular for recreational activities among local tourists. Inshore fishing activities are common along the coastal water. Various local restaurants are available at the beach within the intertidal areas. The beach gradient was 23º.

Plate 3. 2 The intertidal area of Teluk Kumbar

3.1.3 Batu Feringghi

Plate 3.3 shows the intertidal area of Batu Feringghi. Batu Feringghi is hugely popular among Penang Island tourists. Its sandy beaches are surrounded by several tourist attractions, flea markets, hotels and restaurants. This area is also popular for water sports activities and beach trampling. Batu Feringghi recorded the least steep gradient, 14º.

Plate 3. 3 The intertidal area of Batu Feringghi

3.1.4 Jelutong

Plate 3.4 depicts the intertidal area of Jelutong. Jelutong is regarded as the most populated and crowded place in Penang Island. The scattered garbage is found at its intertidal area. A jetty, aquaculture cages and on-going constructions are also notified nearby the intertidal area. The power plant, landfill and sewage treatment plant are located across and around the sampling site. Due to the above-mentioned situations, Jelutong is considered as the most anthropogenically impacted site compared to others. The beach gradient was 40º.

Plate 3. 4 The intertidal area of Jelutong

3.1.5 Teluk Aling

Plate 3.5 illustrates the Teluk Aling sampling site. Teluk Aling is located at the Penang National Park (PNP) and the site for the Centre for Marine and Coastal Studies (CEMACS) of USM. The shoreline of Teluk Aling is made up of rocky shores, very steep with long and flat sandy beaches. Teluk Aling is considered least disturbed and therefore, was selected as a reference site for this study. The gradient of Teluk Aling beach was 15º.

Plate 3. 5 The intertidal area of Teluk Aling

3.2 Sampling of Foraminifera

Samples of sediment were randomly collected at the uppermost layer by using a shovel. Five replicates were collected at each zone (i.e. lower, middle and upper zones) along the intertidal area in a 1 x 1 m^2 quadrat. Random numbers were generated using “Research Randomizer” where three sets of numbers were created and each set containing five random numbers (Figure 3.2). In total, 375 samples were collected throughout the sampling period.

Lower zone is an area that is constantly exposed to the air with almost no vegetation (Schlacher et al., 2008). The middle zone is also known as a soft-bottom sediment. The zonation in the sandy beach is determined by the water level and the period of the zonation exposed to the air.

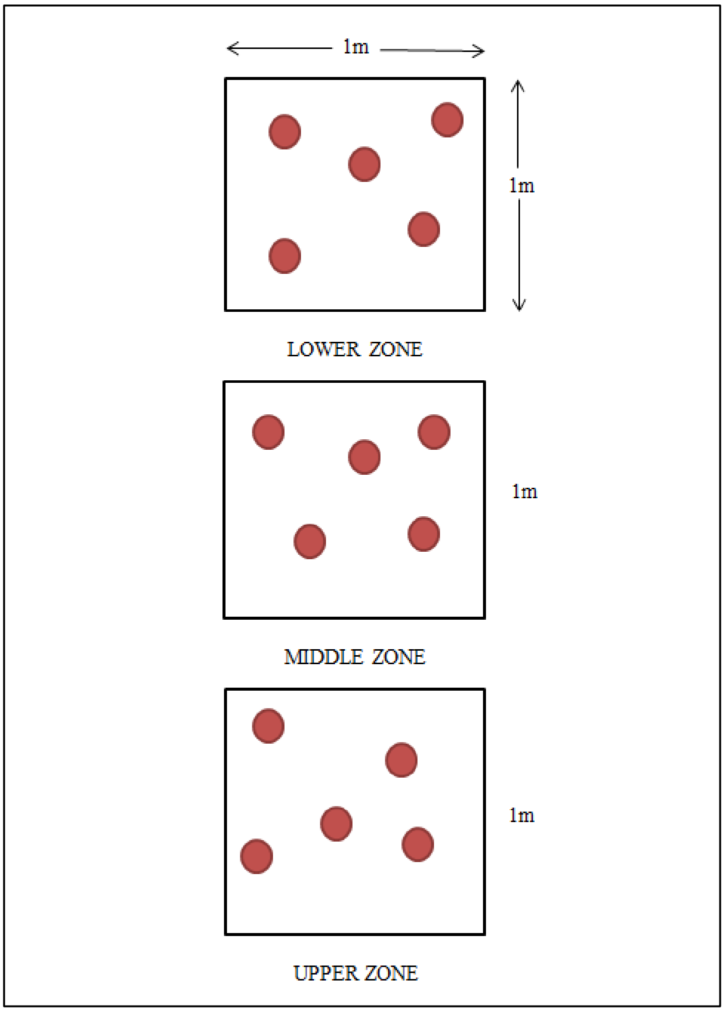

Figure 3.2 the 1m^2 quadrat that placed in each zonation

The distance between each zone varied according to the length of the beach. The upper zone refers to the zone submerged by water when the water level is high. This zone is largely exposed to the air than being submerged by the seawater. Meanwhile, the middle zone is the zone exposed to the air and being submerged by the seawater at an equal time. The lower zone, on the other hand, is continuously submerged by the seawater at all time and is rarely exposed to the air, except during the extremely low tide i.e. mean lower low water.

All samples were immediately preserved according to the method proposed by Eleftheriou and McIntyre (2005). Each sample was subsequently fixed with a pre-mixed solution containing ethanol and Rose Bengal stain. The ethanol was used to fix the foraminifera samples in the sediment, while, Rose Bengal was used to stain the cytoplasm of the foraminifera. This method was applied to distinguish the living foraminifera from the dead. All samples were brought to the laboratory for further analysis.

3.2.1 Counting and Identification of Foraminifera

An 8 cm^3 of subsamples were taken out from the preserved sediment samples to analyze and identify the foraminifera by using a 2 cm x 2 cm x 2 cm cube. Later, the samples were filtered through a stacked sieve with different mesh sizes, ranging from 1 mm to 63 μm mesh. The samples were counted up to at least 300 individuals in each replicate (Murray 2006) and were observed under a dissecting microscope with 2.4x to 240x magnification (Meiji EMZ 57378, Japan).

The abundance of foraminifera was measured by dividing the number of individuals by the volume of the subsamples (8cm^3). The unit of foraminiferal abundance was represented by individuals/8cm^3.

Identification of foraminifera was done based on their chamber arrangement and aperture according to Leoblich and Tappan (1987), Sen Gupta (2003) and Debenay (2012).

3.3 Sediment Analysis

Sediments were scooped using a shovel and were transferred into a labelled plastic bag. The collected sediments were brought back and analysed for organic matter and particle size analysis.

3.3.1 Organic Matter Analysis

The organic matter in the sediment samples was determined by using loss on ignition (LOI) method (Bale & Kenny 2005). Approximately, the sediment samples (5g) were taken and placed into Petri dish and were dried in an oven at a temperature of 105°C. The dry weight was determined using analytical balance (AND EJ410 Compact scale).

Then, the dried samples were transferred into porcelain crucibles and placed into a muffle furnace at a temperature of 550°C for 3 hours to allow complete ignition. After the ignition, the samples were left to cool down overnight and were weighed the next day to determine the weight of ashes. The percentage of organic matter was calculated using the following formula:

% Organic matter = (Dry weight – Ash weight) / Dry weight x 100

3.3.2 Particle Size Analysis

This analysis was done based on Bale & Kenny (2005). About 25 g of sediment samples were dried for 24 hours in an oven at a temperature of 105°C. The dried sediment samples were placed in 500 ml beaker. Sodium hexametaphosphate $(NaPO_3)_6$ solution was prepared by adding 6.2 g of $(NaPO_3)_6$ to 1L of distilled water. Later, 250 ml of distilled water was added to 10 ml of $(NaPO_3)_6$ solution. This solution was subsequently poured into the beaker containing the sediment samples and was mixed together. The mixture was stirred using a glass rod to break up the sediments and was left overnight. The function of $(NaPO_3)_6$ is to separate the sediments. The sediment was subsequently poured onto a stack of sieve arranged in five different sizes, namely 1000 µm, 425 µm, 250 µm, 125 µm and 63 µm based on Wenworth scale (Table 3.2). The residue of each sieve was dried for 24 hours and the fraction of the residue was weighed using an electronic balance. The percentage of each fraction was calculated using a formula below:

$$\text{Particle size percentage (\%)} = \frac{\text{Final weight} - \text{Initial weight} \times 100}{\text{Initial weight}}$$

Table 3. 2 Classification of particle size according to Wentworth (1922).

Sieve size	**Sediment Classification**
>2000µm	Gravel
2000µm-1000µm	Very coarse sand
1000µm-710µm	Coarse sand
710µm-425µm	Mixture of coarse and medium sand
425µm-250µm	Medium sand
250µm-125µm	Fine sand
125µm-63µm	Very fine sand
<63µm	Silt and clay

3.4 Environmental Parameters

Environmental parameters such as beach gradient, soil pH, soil salinity and soil temperature were measured *in situ* at each sampling site. Readings were taken three times at each sampling site.

Soil salinity was measured by using Digital 0-53% Brix Refractometer: Atago PAL-1. The salinity was obtained using an interstitial technique of which the sediment was inserted into the syringe and was forcefully pressed until the water flushed from the syringe.

The soil pH was determined using a pH meter (Ohaus Starter Portable pH Meter - ST300) and the soil temperature was taken using a waterproof soil thermometer with an accuracy of ± 0.1^0 C by placing the probe into the sediments at 10 cm depth. Readings were taken three times after five seconds of probe placement. The slope or gradient of each intertidal area was measured using a clinometer to investigate the beach profiles i.e. changes of beach gradient from the shore to the sea. Size of the beach at all sampling sites was determined by these gradient data.

3.5 Statistical Analysis

3.5.1 Analysis of Variance (ANOVA)

Three-way ANOVA was carried out to analyse all parameters by comparing the means of dependent and independent variables and to find the significant difference of dependent variables (i.e. sampling sites and months). Three independent variables were selected i.e. sampling months, sampling site and zones. The samples were normally distributed with homogenous variances. Data were analysed using the IBM SPSS Statistic version 21 at a significant level of $p = 0.05$.

3.5.2 Pearson's Correlation

Pearson's correlation coefficient with two-tailed test was used to analyse of the correlation between two variables (i.e. foraminifera assemblages and environmental parameters) using IBM SPSS statistic version 21.

3.5.3 Cluster Analysis

Both foraminifera and environmental data were used for cluster analysis.The cluster analysis was used to organize the data in a simplified manner and was classified according to its similarity/dissimilarity. This study used a hierachical cluster with Ward's method to measure the similarity distance between cases (i.e. foraminifera assemblages and environmental parameters).

3.6 Species Diversity Indices

Species diversity indices using Shannon–Weiner index were performed to quantitatively analyse the species present in the samples as well as the distribution of individuals among the species. By performing the diversity indices, both species richness and evenness were incorporated into a single value (Ludwig & Reynolds 1988).

The Shannon – Wiener equation is as followed:

$$H' = -\sum_{i=1}^{s} pi \ \log_e pi$$

With, H' = Shannon – Wiener diversity index

pi = the proportion of *i*th species

log *e* = the natural logarithm of *pi*

S = the number of species in the community